Special Protocol for extraction of micro RNA from Serum/Plasma Exosome

Read the Important Note before starting the following steps.

HINT: Preheat Release Buffer to 65°C for step 12.

1. Add 200 µl Lysis Buffer into the tube containing up to 200 µl exosome sample.

IMPORTANT! If the initial sample volume exceeds 200 µl, the samples will need to be processed in multiple loads due to limitations

of buffer capacity.

2. Vigorous mixing by vortexing thoroughly. Incubate at room temperature for 10 minutes.

3. Add 20 µl 2M NaOAc, pH 5.2.

4. Add 180 µl water-saturated phenol and 40 µl chloroform into the tube, vortex vigorously for 2 minutes.

5. Centrifuge at 12,000 rpm for 3 minutes. Transfer the upper phase carefully into a new 1.5 ml centrifuge tube.

IMPORTANT! Avoid picking up any of the interphase or organic layer when transferring the aqueous phase (upper phase).

6. Add absolute ethanol (2.35 × volume of upper phase) to the upper phase and mix well by shaking vigorously.

-- If the upper phase volume is 200 µl, add 470 µl of absolute ethanol to upper phase. The final ethanol concentration of whole

mixture will be 70%.

7. Place a RNA Column in a Collection Tube and transfer the ethanol-added mixture to the RNA Column. Incubate for 1 minute.

8. Centrifuge at 12,000 rpm for 30 seconds (miRNA bound to the column membrane).

9. Add 200 µl Wash Buffer 2 (ethanol added). Incubate for 1 minute.

10. Centrifuge at 12,000 rpm for 1 minute to completely remove the residue liquid.

11. Put the RNA Column to a new 1.5 ml centrifuge tube.

12. Add 30~50 µl Release Buffer (preheated to 65°C) to the center of column. Incubate for 3 minutes.

13. Centrifuge at 12,000 rpm for 3 minute to recover miRNA.

14. Store the isolated miRNA at -80°C